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GROWTH AND DEVELOPMENT OF THE SALIVARY GLAND CHROMOSOMES IN SCIARA

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The chromosomes in the larval salivary glands of Diptera are unique in their enormous size, in the remarkable regularity and complexity of their visible detail and in the intimate fusion of homologs. The present investigation of *Sciara* is an attempt to follow these characteristics during the transformation of the ordinary small somatic chromosomes into the giant banded salivary chromosomes. Two methods were used. In the first, the growth of chromosomes, nucleus, cell, salivary gland and larva was measured *in vivo* throughout development. In the second, a careful study was made of the morphology of chromosomes fixed at each stage of development, in an attempt to correlate growth with morphological change. A detailed account of the investigation will be published elsewhere.

At room temperatures *Sciara* requires about a month for development from egg-laying to the emergence of the imago. Its four instars and pupation begin, respectively, at about 6.5, 10, 12.5, 15 and 24 days.

The salivary gland attains its definitive number of cells soon after it arises in the embryo within the egg, and its subsequent growth is due entirely to increase in cell size. In nucleus, cell, gland and larva, growth proceeds at a moderate rate up to the beginning of the second instar, then more rapidly until about the middle of the fourth instar, then more slowly until pupation, at which point visible degeneration begins and the size of each decreases.

In the early embryonic gland homologous chromosomes are somatically paired, but not fused in synapsis.[1] Each homolog is present as a relatively short and very slender thread lying parallel to its mate and about 0.4 μ distant, measured axis to axis. Each thread bears a small number of chromomeric enlargments corresponding to those on its homolog. Somewhat later each homolog splits into two threads and these sister strands are joined to each other by delicate cross-connections between some of the homologous chromomeres.

Shortly before the larva hatches from the egg the doubled homologs become more closely associated and begin to twist or twine about each other, caduceus fashion. At the same time the cross-connections between sister strands become heavier, obscuring the original chromomeric structure, except in stretched regions, and foreshadowing the "banding" of the typical salivary chromosome. The bipartite homologs continue to draw closer to one another, and the twisting, as it grows tighter, forms a helical coil which is composed of the two homologs (each double) lying parallel to each other and apparently in contact. In many nuclei of this age each coiled pair of homologs is distinctly separate from the others and all are oriented (polarized) toward the same region of the nucleus, so that the gross orientation resembles that in late telophase. As soon as the homologs come in contact a fusion between homologous regions begins, in the sense that the median line dividing them gradually disappears. This fusion may properly be considered the beginning of the synapsis of salivary mates. The earliest synapsed salivary chromosome thus is flattened, four-stranded and helically coiled. Synapsis is substantially completed during the first instar, which is very much earlier than the stage at which, according to Painter,[2] it occurs in *Drosophila*. By the end of the first instar each synapsed pair of homologs appears as a slender cylindrical, much elongated strand, considerably convoluted but only irregularly coiled. They stain lightly, show diffuse cross-bands at intervals and are considerably longer in proportion to their diameters than they are in old larvae.

Early in the second instar the chromosomes begin to increase in diameter and the banding becomes more pronounced. By the time the late second instar has been reached their structure, except for smaller size and fewer bands, is essentially that seen in old larvae. In this stage also, a regular coiling of each synapsed chromosome pair as a unit again begins to become prominent.

During the third instar the chromosomes increase in diameter at a constant rate. Midway through this instar the coiling reaches its maximum, and in favorably smeared preparations or in Feulgen mounts, each chromosome is seen to be coiled into a fairly tight uniform helix which superficially resembles the metaphase spirals seen in liliaceous chromosomes. In the late third instar the coiling begins to relax, and the chromosomes become progressively straighter throughout the fourth instar.[3]

The fourth instar is the period of greatest growth of the chromosomes. In reference to the development of the banding, the evidence indicates that the first bands which appear represent the heaviest bands of the definitive chromosome and remain relatively unaltered during development except to darken or to separate into doublets. The new, light bands, which become visible as development proceeds, appear in the lengthening spaces between the heavier bands, rather than split off from them. In the late fourth

instar there are usually visible certain small regions where the homologs are partially or completely non-synapsed, but no separation of the synapsed chromosome into more than two units has been observed. There seems to be in *Sciara* no visible differentiation of the definitive chromosome into four "bundles of chromonemata" of the sort reported in *Drosophila* and *Chironomus* by Tiniakov,[4] and in *Simulium* by Painter and Griffin.[5]

During the pupal stage the larval salivary gland undergoes histolysis. The main outlines of the degeneration of the chromosomes seem to be as follows: decrease in volume due to loss of the achromatic ("matrix") material; the collapse of enlarged regions to form heavy-walled vesicles; the persistence of very heavy bands; and the disappearance of delicate bands and fine detail.

If the average nuclear volume in the early salivary gland of the embryo is expressed as unity, calculation shows that the salivary gland nuclei in larvae at the time of pupation are about 1750 times as large. The curve of increase in nuclear volume during development is approximately exponential from about 4.5 days to 17 days (i.e., log nuclear volume plotted against time is a straight line) and can be superimposed rather closely upon a curve of the general form $y = 2^n$ up to the value $n = 10$. This indicates that nuclear volume doubles every 1.25 days during the period from 4.5 to 17 days. The rate of increase after the 17th day is progressively less until pupation. Doyle and Metz[6] have shown that in the living condition the full-grown salivary gland chromosomes of *Sciara* occupy at least 90 per cent of the volume of the nucleus. It is therefore not unreasonable to assume that the chromosomes increase in volume at approximately the same rate as the nucleus. This does not necessarily mean that there is a regular doubling of some internal structure of the chromosomes every 1.25 days, as their growth (increase in volume) could conceivably be due, partly or entirely, to some other cause, such as intake of fluid.

Chromosome volume cannot be computed directly because it is impossible to measure accurately the lengths of the chromosomes during the early stages. The growth in volume per unit length, however, is given at once by the cross-sectional area at different stages, and this, when calculated from the measurements of chromosome diameter, proves to increase about 75-fold between synapsis and pupation. During the same period nuclear volume increases 300-fold. Assuming that the chromosomes occupy the same percentage of nuclear volume throughout larval development, the above results mean that chromosome length increases about 4-fold $\left(\frac{300}{75}\right)$ during this period. The total length of the mature relaxed salivary chromosome group is around 800 μ, and that of the metaphase group around 10 μ. The 80-fold difference between these lengths is thus made

up of the 4-fold increase subsequent to synapsis and a 20-fold increase previous to synapsis.

The measurements of Belling[7] on *Lilium* show that the "fully-extended" leptotene chromonema is 10 times the length of the metaphase group. Measurements made by the writer from the drawings of Kaufmann[8] indicate that the difference is of the same order of magnitude in *Drosophila*. Kaufmann[9] and others have suggested that the heavily staining chromomeric granules seen in the spireme stages of some ordinary chromosomes represent regions where the connecting thread (chromonema) remains tightly coiled in a tiny spiral (not to be confused with the coiling of the chromosome as a whole, described above). Genetic evidence makes it probable that the definitive salivary chromosome is comparable to a chromonema entirely uncoiled and extended at full length. It is questionable whether mere "uncoiling" of chromonemata can account for the enormous (80 to 100-fold) difference in length between the metaphase and definitive salivary chromosomes, as assumed by Muller.[10] Rather, it seems more reasonable to assume that the great increase in length is due partly to uncoiling and partly to actual growth in length of the chromonemata, provided, of course, that the chromosomes are indeed made up of chromonemata. It is difficult to ascertain what proportion of the increase is due to either of the above factors, but it is interesting to note that previous to and during synapsis (first instar) the chromosomes increase greatly in length, but little in diameter. This period might, therefore, be one in which the chromonemata lose their hypothetical coiling and become completely extended. In the latter part of the fourth instar, when specific regions of the chromosomes can be recognized, it is reasonably clear that increase in length is due to actual growth of the chromosomes, elongation of the interband portions accounting for most of the increase. The fact that the chromosomes elongate appears to vitiate the calculations of Hertwig[11] concerning the number of chromonemata present in the salivary chromosomes, since in his work only the nuclei were measured, and no allowance was made for increase in nuclear volume by chromosome elongation.

Koltzoff[12] and Bridges[13] have advanced the hypothesis that the definitive salivary chromosomes are probably bundles of gene-strings (chromonemata) derived by repeated longitudinal division of the chromonemata present in ordinary somatic chromosomes. These investigators, in addition, have reported that in *Drosophila* the number of granules visible in a band frequently appears to be a multiple of 2, most often 8, 16 or 32. On this view the number of chromonemata present would be equal to or greater than the number of granules separately visible on any band. Metz and his coworkers,[14] while not disputing the compound nature of the salivary chromosomes, have raised serious objections to the view that there is any identity

of the "striations" which connect granules along the lengths of the chromosomes, with the individual genonemes.

In order to ascertain the relation between the number of chromonemata theoretically to be expected on the basis of the above measurements, and the number of granules and striations visible in the salivary chromosomes, let us make the following assumptions: (1) The synapsed salivary chromosomes are solid cylinders of circular cross-section, having the same type of structure throughout a given transverse section.[15] (2) The chromosomes increase in cross-sectional area by longitudinal multiplication of chromonemata. (3) All chromonemata formed during development are the same size and distance apart as the original threads at synapsis.[16] If we accept these assumptions tentatively, the data on cross-sectional area of the chromosomes show that since each chromosome (pair of homologs) contains 4 visible threads at synapsis (area = 1), one would expect that there would be approximately 300 chromonemata visible in each full-grown chromosome (area = 75). Observation, however, does not support this expectation. The maximum number of granules clearly visible in any band was around 20 (Metz reports counting about 30). Similarly, at every stage of postsynaptic development the observed number of granules per band is very much less than the number of chromonemata to be expected if the above assumptions are correct, although many more granules than are seen should be easily visible if present. The observations, however, do not preclude the possibility that the visible striations represent compound bundles of incompletely separated chromonemata.

In conclusion, the discrepancy between observation and calculation means either that one or more of the above assumptions is wrong or that the finest visible striations and granules in the mature salivary chromosomes do not correspond to single chromonemata of the sort clearly seen in some ordinary chromosomes.

The author gratefully acknowledges his indebtedness to Doctor Calvin B. Bridges, who offered much helpful advice during the course of the investigation and carefully confirmed the main observations, and to Professor C. W. Metz, who suggested the problem and furnished the material.

[1] Painter (*Jour. Hered.*, **25**, 465, 1934) and Dobzhansky and Tan (*Zeits. f. ind. Abst.*, **72**, 88, 1936) have drawn similar distinctions between pairing and synapsis.

[2] Painter, T. S., *Science*, **78**, 585 (1933).

[3] The progress of coiling in *Sciara* does not appear to agree with that reported for *Drosophila*, in which Koller (*Proc. Roy. Soc.* (London), Ser. B, **118**, 371, 1935) maintains that the chromosomes are progressively *un*-coiling from the earliest stages. Koller, however, studied mainly "developing" cells in glands from *old* larvae.

[4] Tiniakov, G. G., *Biol. Zhurnal*, **5**, 753 (1935).

[5] Painter, T. S., and Allen B. Griffin, *Genetics*, **22**, 202 (1937).

[6] Doyle, W. L., and C. W. Metz, *Proc. Nat. Acad. Sci.*, **21**, 75 (1935); *Biol. Bull.*, **69**, 126 (1935).

[7] Belling, John, *Univ. Calif. Publ. Botany*, **14**, 335 (1928).

[8] Kaufmann, Berwind P., *J. Morph.*, **56**, 125 (1934).

[9] Kaufmann, Berwind P., *Am. Nat.*, **65**, 280 (1931).

[10] Muller, H. J., *Am. Nat.*, **69**, 405 (1935).

[11] Hertwig, Gunther, *Zeits. f. ind. Abst.*, **70**, 496 (1935).

[12] Koltzoff, Nic., *Science*, **80**, 312 (1934).

[13] Bridges, C. B., *Am. Nat.*, **69**, 59 (1935).

[14] Metz, C. W., *Jour. Hered.*, **26**, 177 (1935); *Ibid.*, **26**, 491 (1935); *Biol. Bull.*, **71**, 238 (1936); *Proc. Nat. Acad. Sci.*, **23**, 137 (1937).

Metz, C. W., and E. H. Gay, *Proc. Nat. Acad. Sci.*, **20**, 617 (1934); *Science*, **80**, 595 (1934).

Metz, Charles W., and Elizabeth Gay Lawrence, *Quart. Rev. Biol.*, **12**, 135 (1937).

[15] This has been observed by Bauer (*Zeits. f. Zellf.*, **23**, 280, 1935), in *Chironomus* and in *Drosophila* (*Proc. Nat. Acad. Sci.*, **22**, 216, 1936) and it appears also to be true in *Sciara*, but Yasui (*Cytologia*, **6**, 330, 1935) and Tiniakov[4] maintain that the salivary chromosomes in *Drosophila* are flat tapes, and Koltzoff,[12] Heitz (*Biol. Zbl.*, **54**, 588, 1934) and Muller[10] that they are hollow cylinders.

[16] In the definitive salivary chromosomes of *Sciara* the visible striations and granules in some regions do appear to be approximately the same size and distance apart as the original chromomeric threads, but in many other regions they are either more or less numerous.

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DEVELOPMENT OF EYE COLORS IN DROSOPHILA: RELATION BETWEEN PIGMENTATION AND RELEASE OF THE DIFFUSIBLE SUBSTANCES

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1. *Introduction.*—Eye buds of the mutants vermilion (*v*) or cinnabar (*cn*) of *Drosophila melanogaster*, implanted into larvae of the mutant white (*w*) develop into eyes which are wild type in color. It has been previously shown that a similar modification of *v* and *cn* eyes implanted into wild type is due to the presence of two diffusible substances (the v^+ and cn^+ substances) in the lymph of the wild type hosts.[2] It must be concluded then that the same substances are present in the *w* mutant and that the partial or total absence of pigment in the mutants of the *w* series is due to the disturbance of a mechanism other than that which leads to the formation of diffusible substances required in the development of the wild type eye color.

It has also been shown[2,3] that eye implants of various mutants can produce and sometimes release the diffusible substances; this can be shown by

implantation of the test eyes into larvae sensitive to one of the substances; if, for example, eye buds that release the v^+ substance are implanted into apricot-vermilion ($w^a\,v$) hosts, there is a modification of the host eyes toward the apricot (w^a) phenotype.

Experiments of this type have shown that the amounts of substances released by the different mutant eyes vary a great deal with the nature of the mutant considered; and there appeared to be a relation between the amount of substance released and the degree of pigmentation of the implant itself.

The primary purpose of the experiments described below has been to establish in a direct way this relation between the release by a given eye of the two substances mentioned and its actual pigmentation. The different w allelomorphs which form a series of increasing intensities of

TABLE 1

RELEASE OF THE v^+ SUBSTANCE BY THE EYES OF FOUR w-ALLELOMORPHS

IMPLANT	HOST	PHENOTYPE OF HOST	NUMBER OF INDIVIDUALS
w	$w^a v$	w^a	8
w^h	"	Slightly lighter than w^a	10
w^{ch}	"	Intermediate between $w^a\,v$ and w^a	7
w^{331}	"	$w^h\,v$	11

TABLE 2

RELEASE OF THE cn^+ SUBSTANCE BY THE EYES OF FOUR w-ALLELOMORPHS

IMPLANTS	HOST	PHENOTYPE OF THE w^a; cn IMPLANT	NUMBER OF INDIVIDUALS
w and $w^a;cn$	$v;cn$	w^a	9
w^h and $w^a;cn$	"	w^a	6
w^{ch} and $w^a;cn$	"	Intermed. between $w^a;cn$ and w^a	3
w^{331} and $w^a;cn$	"	$w^a;cn$	8

pigmentation, appeared to fit our purposes particularly well and have, therefore, been used in the present experiments.

2. *Release of the v^+ substance.*—The release of v^+ substance by four members of the w series (w, honey (w^h), cherry (w^{ch}) and white-331 (w^{331}) used as implants, has been evaluated by their effect on $w^a\,v$ hosts, sensitive to the v^+ substance. The results of this experiment are shown in table 1.

These data suggest that the release of v^+ substance is inversly proportional to the intensity of pigmentation of the eye used as the source of the substance.

3. *Release of the cn^+ substance.* The release of the cn^+ substance by the same w allelomorphs has been measured by simultaneous implantation of these test eyes and of $w^a;cn$ eyes (sensitive to the cn^+ substance) into

v;cn hosts, known to be lacking both the v^+ and the cn^+ substances. The results of these experiments are given in table 2.

These data indicate an inverse proportionality between the release of the cn^+ substance and the pigmentation of the eye used as the source of the cn^+ substance.

It should be pointed out that in the experiments cited, the $w^a;cn$ eye itself constitutes a source of a small amount of v^+ substance[4,5] which can be converted to cn^+ substance by the other implant. However, it is obvious that the amount of this v^+ substance is small and constant enough, so that it does not materially affect the result.

4. *Conversion of the v^+ substance to the cn^+ substance.*—The $w^a;cn$ eyes have been implanted into a series of five double recessives of *v* with an allelomorph of *w*. This series is: white-vermilion (*w v*), apricot-vermilion (w^a *v*), blood-vermilion (w^{bl} *v*), coral-vermilion (w^{co} *v*) and white-331-vermilion (w^{331} *v*). These five double recessives also show an increasing intensity of pigmentation, the difference between w^{co} *v* and w^{331} *v* being extremely small at 25°C. Because homozygous for *v*, they are unable to produce any of the two substances considered, but they can convert the small amount of v^+ substance supplied by the $w^a;cn$ implant to cn^+ substance. The degree of modification of the $w^a;cn$ implant toward w^a serves to evaluate the relative amount of substance converted.

TABLE 3

CONVERSION OF THE v^+ SUBSTANCE TO cn^+ SUBSTANCE

IMPLANT	HOST	PHENOTYPE OF IMPLANT	NUMBER OF INDIVIDUALS
$w^a;cn$	*w v*	w^a	1
"	w^a *v*	w^a	7
"	w^{bl} *v*	Intermed. between $w^a;cn$ and w^a	6
"	w^{co} *v*	"	9
"	w^{331} *v*	$w^a;cn$	6

It can be seen from table 3 that the more pigment contained in the host eyes, the less the effect on the implant. In other words, the host received a constant amount of v^+ substance from the implant and converted a fraction of it to cn^+ substance; this fraction appears to be inversely proportional to the intensity of pigmentation of the host eyes.

5. *Release of the cn^+ substance in the Presence of an Excess of v^+ substance.*—It is interesting to compare the experiments described in paragraph 3 with an experiment involving the implantation of eyes of the *w* series into $w^a;cn$ hosts. The eyes serving as the source of the substance and those serving as receptors are the same in the two experiments. The difference between the two experiments lies in the fact that in the experiments reported above the eye serving as the source of the cn^+ substance

(*w* allel) received from the $w^a;cn$ implant only a very small amount of the v^+ substance, while in the experiment to be reported below (implantation of eyes of the *w* series into $w^a;cn$) the eye serving as source of cn^+ substance (the same *w* allel) is placed in a medium containing an excess of v^+ substance. Table 4 shows that the extreme effect (complete change to w^a), which has previously been produced only by *w* and w^h implants, is obtained now by implantation of *w*, w^h and w^{ch}; and that w^{331} produces here a clear effect, while it produced no effect in the experiments described in paragraph 3.

It is clear, then, that the ability to convert the v^+ substance into the cn^+ substance, as measured by the release of the latter, also depends on the

TABLE 4

RELEASE OF THE cn^+ SUBSTANCE IN THE PRESENCE OF AN EXCESS OF THE v^+ SUBSTANCE

IMPLANT	HOST	PHENOTYPE OF HOST	NUMBER OF INDIVIDUALS
w	$w^a;cn$	w^a	11
w^h	"	"	9
w^{ch}	"	"	11
w^{331}	"	Intermed. between $w^a;cn$ and w^a	10

TABLE 5

RELEASE OF THE v^+ SUBSTANCE BY EYES HOMOZYGOUS FOR EITHER *bw;cd* OR $pn^2;cd$ COMPARED WITH THAT OF *bw*, pn^2 AND *cd* EYES

IMPLANT	HOST	PHENOTYPE OF HOST	NUMBER OF INDIVIDUALS
bw	w^a *v*	w^a *v*	*
cd	"	"	*
pn^2	"	"	*
bw;cd	"	Intermed. between w^a *v* and w^a	13
$pn^2;cd$	"	"	7

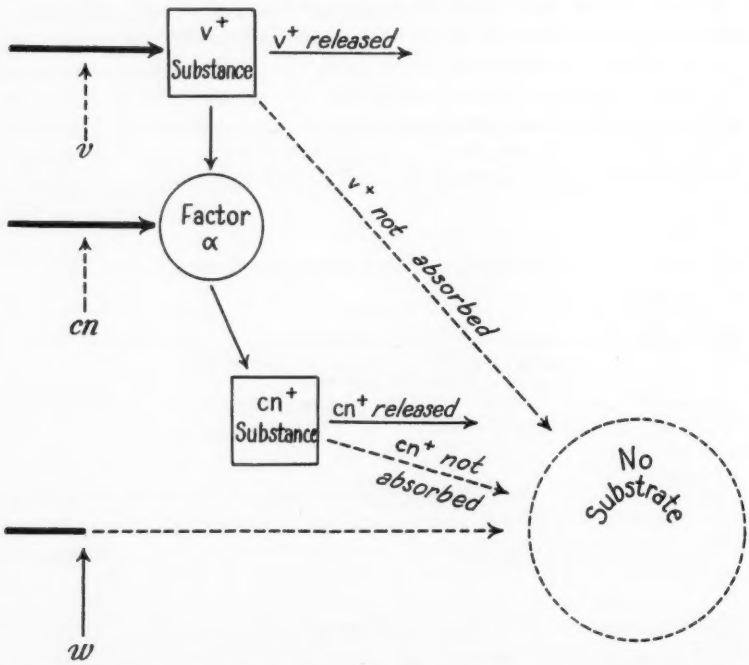
* Data of Ephrussi and Beadle.[3]

actual pigmentation of the eye. In paragraph 4 the conversion was brought about by a *v* eye, while here a v^+ eye causes the same transformation. This shows that the relation which we have established holds in the two cases or that, in other words, an eye which itself produces the two substances can nevertheless transform a certain amount of v^+ substance supplied from another source.

6. *Does the Relation between Release of the Diffusible Substances and Pigmentation Apply to Other Cases than the w Mutants?*—Before considering the observed relation between intensity of pigmentation and release of substances as proved, we had to eliminate a possible objection. It had to be demonstrated that the release of substances is not associated in a purely fortuitous manner with the quantity of pigment shown in the eyes of the

different w allelomorphs. To do this the release of v^+ substance by the eyes of two double-recessives has been examined. We have chosen the mutants brown (*bw*), cardinal (*cd*) and prune-2(pn^2) because the double recessives *bw;cd* and pn^2;*cd* are very light as compared with the simple mutants and because none of those simple mutants release the v^+ substance when tested by implanation into w^a *v* flies.[3]

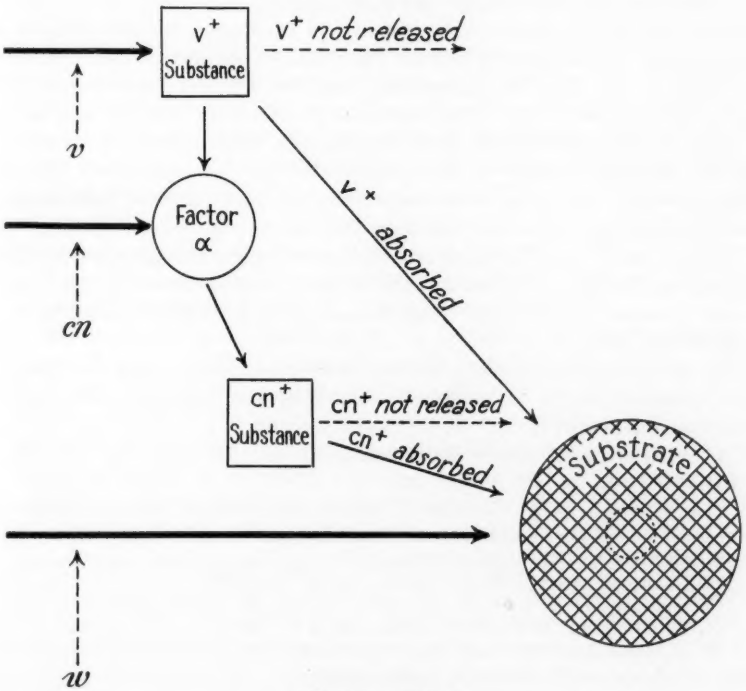
The results of these experiments are shown in table 5, from which one can see that both double recessives do release the v^+ substance. This is in



agreement with the assumption that there is a causal relationship between the release of the substances by a given eye and its actual pigmentation.

7. *Discussion.*—As suggested by Ephrussi and Beadle,[6] the amount of substance released by a given eye can be considered as equal to the difference between the amount produced and the amount used by the eye itself. Experiments constituting additional evidence which gives support to this view are published elsewhere[7] and, therefore, are not considered here. It will be mentioned only that these experiments have shown that an eye producing v^+ substance will not release the substance as long as its own

requirements are not satisfied. The same rule applies to the release of the cn^+ substance as well as to the transformation of the v^+ substance into the cn^+ substance when this transformation is evaluated by the release of the cn^+ substance.



FIGURES 1 AND 2

Schematic representation of the relation between release of diffusible substances and eye-pigmentation. Vertical arrows above symbols indicate points at which the independent formation of the v^+ substance, of the factor α and of the substrate, may be interrupted by the intervention of the mutant genes (solid arrows indicate reaction is taking place). Further explanation in text.

On the basis of these facts, taken together with the results of the experiments reported in the present paper, the following scheme of action of the v^+ and cn^+ substances in the process of pigment formation is proposed.

It will be recalled that Beadle and Ephrussi[2] have advanced the following scheme for the formation of the v^+ and the cn^+ substances:

$$\longrightarrow v^+ \longrightarrow cn^+$$

Figures 1 and 2 show the way in which we propose to complete this scheme. It is known that the v^+ substance is necessary for the formation of the cn^+ substance. We assume that the formation of the cn^+ substance results from the interaction of the v^+ substance with what we call "factor α." As is indicated by the vertical dotted arrows, the formation of the v^+ substance and that of the "factor α" (and, therefore, of the cn^+ substance) can be interrupted independently of each other by the intervention of the mutant genes *v* and *cn*. In order to account for the relation between the intensity of pigmentation and the release of substances, we postulate another factor: the "substrate," which we consider is a precursor of the pigment and which, therefore, is variable. The formation of the substrate is more or less inhibited by the intervention of the *w* allelomorphs. The transformation produced by the v^+ and cn^+ substances would be brought about by their action on the substrate.

In the case of eyes with little or no substrate (case of the mutant *w* which blocks the formation of pigment) the v^+ and cn^+ substances would not be used or would be used only in small amounts and, therefore, could be released (Fig. 1).

In the case of eyes rich in substrate (heavily pigmented eyes) the diffusible substance would be entirely consumed by the substrate and, therefore, not released (Fig. 2).

All intermediates can be easily imagined between these two extreme cases.

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[2] G. W. Beadle and B. Ephrussi, *Genetics*, **21**, 225–247 (1936).

[3] B. Ephrussi and G. W. Beadle, *Bull. Biol. Fr. Belg.*, **71**, 75–90 (1937).

[4] G. W. Beadle and B. Ephrussi, *Genetics*, **22**, 76–86 (1937).

[5] B. Ephrussi and G. W. Beadle, *Bull. Biol. Fr. Belg.*, **71**, 54–74 (1937).

[6] B. Ephrussi and G. W. Beadle, *Genetics* (in press).

[7] B. Ephrussi and S. Chevais, *Bull. Biol. Fr. Belg.* (in press).

AUTOSOMAL LETHAL MUTATION FREQUENCIES IN DROSOPHILA

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The aim of developing a technique for measuring the basic rate of mutation in *Drosophila*, and then discovering how this rate was modified by various environmental influences, was pursued by Muller[1] almost single-handed for many years. His first method, finally described in detail in 1928, was designed to cause accumulation of chromosome II lethals through a number of generations of a "balanced lethal" stock. The frequencies of lethals found in two similar lines carried through the interval at different temperatures, divided by the number of generations, were compared, and the difference, though statistically not significant, suggested a doubling of the rate of mutation for a 10° rise in temperature.

The successful development of the *Cl B* method of revealing lethals in chromosome I, and the marked increase in mutation frequencies produced by suitable x-ray doses led to the general belief that this method was sufficient to decide the questions originally set by Muller. For other agents in the normal enviornment, as increased temperature, varying humidity, chemical factors in the food, this expectation has not been completely realized. (Cf. Goldschmidt, Jollos, Plough and Ives,[2] Timofeeff-Ressovsky,[3] Ssackarow.[4]) It appears, for instance, that a 12-hour exposure of larvae to a temperature of 36.5° causes an increase in mutation frequency of from three to six times. However, the actual numbers of chromosomes showing mutations forms so small a fraction of the total, that some doubt continues to exist as to the significance of the recorded results. This has been further intensified by the discovery (Demerec,[5] Plough and Holthausen[6]) that marked differences in the frequencies of *X*-lethals occur in different stocks. Thus Timofeeff-Ressovsky found about 0.2 per cent of spontaneous *X*-lethals in the controls of a large series, while Demerec, and Child and Plough[7] found in excess of 1.0 per cent in Florida stocks. Thus, it appears that some method giving larger numbers of mutations is needed in order completely to dispel all doubt that other environmental agents than x-radiation cause increases in mutation frequency over the basic rate, or indeed to establish beyond question that a basic rate of spontaneous mutation occurs in a constant optimal environment.

It is this problem which we have been attempting to work out during the past two years, and concerning which we have already made two reports at successive meetings of the Genetics Society of America. Muller has continued his interest in the problem and suggested the independent

investigation by similar methods recently published by Berg.[8] The method constitutes a return to Muller's original method cited above, namely, the determination of the frequencies of lethal mutations in the large autosomes II and III. The method for the simultaneous testing of lethals in these two chromosomes was worked out and the stocks put together by one of us (Child). The scheme of matings is charted in figure 1. Tests of the numbers of lethals in one II and one III chromosome of generation 1 are made in generation 4. All lethals which appeared were subsequently checked and located with respect to *Bl* (II) and *D* (III).

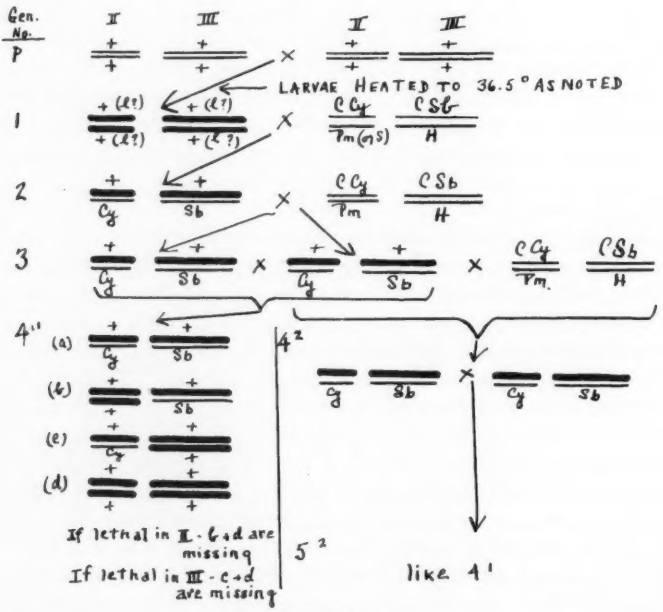

FIGURE 1

Scheme for detecting the number of lethal mutations per generation in Chromosomes II and III.

Preliminary experiments with this method made early last year showed that a Florida stock (isogenic No. 10) made completely homozygous about two years earlier gave a much higher percentage of lethals in both II and III than had ever been recorded under normal conditions for chromosome I. For that reason, it appeared that the method offered a favorable scheme for getting significant data on the spontaneous mutation rate as well as on the effects of various temperatures and other environmental agents.

We therefore selected one normal stock derived from the above prelimi-

nary experiment which had been rendered isogenic a second time and showed no lethals nor semi-lethals. The main experiment began in October, 1936, about six months (±15 generations) after the initial preliminary test, during which the selected + line had been carried in mass cultures. The first broods from each of 50 pair matings were reserved as controls and kept at 25°. The second broods were subjected to 36.5° for 12 or 24 hours as shown in the table. The flies were kept in our new constant temperature room throughout the experiments. Here the temperature varies not more

TABLE 1

AUTOSOMAL LETHALS IN *Drosophila* FOLLOWING EXPOSURES TO HEAT. CHILD AND PLOUGH, 1936

	DAY AFTER LAYING WHEN EXPOSED TO 36.5°	TOTAL II AND III CHROMOSOMES TESTED	NO. II LETHALS	PERCENTAGE II LETHALS PER CHROMOSOME	NO. III LETHALS	PERCENTAGE III LETHALS PER CHROMOSOME
		Controls continuously at 25°				
		92	5	05.4	21	22.8
		Experiment I—25° except for 12-hour exposure to 36.5°				
	2	28	4	14.3	12	42.9
	3	34	3	08.8	16	47.0
	4	22	2	09.1	5	22.7
	5	20	1	05.0	8	40.0
	6	24	1	04.2	4	16.6
	7	23	6	26.0	10	43.5
Total	2–7	151	17	11.3	55	36.4
		Experiment II—25° except for 24-hour exposure to 36.5°				
	3	63	12	19.0	31	49.2
	4	38	2	05.3	15	39.4
	5	9	1	11.1	7	77.8
	6	17	0	00.0	3	17.6
	7	9	0	00.0	5	55.6
Total	3–7	136	15	11.0	61	44.8

than 0.2° in any compartment and the moisture is kept constant within 5% relative humidity.

The results of the tests are given in table 1. It will be seen from the control values that 05.4 per cent II chromosomes showed lethals while for chromosome III the result was much higher—22.8 per cent. The heated larvae showed marked differences depending on the day of larval life when they were heated. In general it appears that the second and the seventh day after laying show the greatest increase in mutation rate, but the comparison of the values between the two chromosomes fails of agreement in certain cases. It is of interest that little difference was observed between

a period of heating of 12 and of 24 hours. The latter exposure sterilizes most of the flies, and so the latter series gave much smaller numbers. The similarity between the results of these two experiments seems to confirm the suspicion that it is the temperature shock rather than the duration of the high temperature which produces an increase in mutation frequency. In general, comparison of the control and experimental totals shows that in a stock kept in mass culture after being made lethal free (1) Autosomal lethals appear with a sufficiently high frequency to be used as significant measures of mutation rates, (2) that chromosome III lethals are about four times more numerous than those in chromosome II, (3) and finally, that temperature shocks in the larval period produce an increase in lethal muta-

TABLE 2

AUTOSOMAL LETHALS IN SUCCESSIVE GENERATIONS OF ISOGENIC STOCKS KEPT CONTINUOUSLY AT 25°

GENERATION TESTED	NO. II CHROMOSOMES TESTED	NO. II LETHALS FOUND	PER CENT II LETHALS PER CHROMOSOME	NO. III CHROMOSOMES TESTED	NO. III LETHALS FOUND	PER CENT III LETHALS PER CHROMOSOME
1 (Oct., 1936)	92	5	05.4	92	21	22.8
2–7	mass cultures not tested					
8 *(Mar., 1937)	51	2	03.9	37	6	16.2
9	68	0	00.0	131	5	03.8
10	106	3	02.8	134	1	00.7
11	65	2	03.1	92	3	03.2
Average 9–11	239	5	02.1	357	9	02.5

* Since March, 1937, the tests for lethals in II and III were conducted separately.

Cl B tests of chromosome I of the same stock at 25° for comparison

	NO. I CHROMOSOMES TESTED	NO. I LETHALS FOUND	PER CENT I LETHALS PER CHROMOSOME
(Apr., 1936)	178	2	01.1

tion frequency which is unquestionably significant. The first of the above statements is amply confirmed by the results of Berg.

Brief consideration of the results given in table 1 will immediately suggest that the control value does not represent the frequency of autosomal lethal mutation per generation. It represents rather the lethal mutations of the generation tested (generation 1) plus the accumulated lethals of the past 15 generations since a test was made. To get at the rate per generation it is necessary to test the *same* chromosomes in successive generations. The 4^2 column of figure 1 shows how that can be done, and it can be continued for as long as the physical endurance of the experimenter lasts. We took up this method on the eighth generation of the non-lethal bearing

line of the experiment just reported (March, 1937). The results have now been tabulated for successive tests of the same chromosomes for four generations more. They are given in table 2. As soon as a lethal was shown that line was retired from the series, so that the number of lethals listed for each of the generations 9–11 is the number which occurred in *that generation only*. This series thus gives us an exact measure of the autosomal lethal rate per generation, as well as an indication of the rate at which lethals accumulate or are eliminated in successive generations in each of these chromosomes.

Comparison of the per cents of II and III lethals per chromosome tested as shown in table 2 reveals that the lethals shown in generation 8 are definitely in excess of those in each of the subsequent generations, especially for chromosome III. This is due to the accumulation of lethals during the preceding six untested generations. Apparently, for reasons yet unknown, third chromosomes bearing lethals are not eliminated as rapidly as are second chromosomes carrying them or there are more mutable "lethal" loci in III. The sum of the per cents of II chromosomes bearing lethals in generations 8–11 is 09.8, which is greater than the number actually shown by generation 1—05.4—after about fifteen generations in mass culture. The difference is an index of the amount of elimination in twelve generations. For the third chromosome the sum of per cents for generations 8–11 is 23.9, which is very slightly in excess of the similar figure for generation 1—22.8. This indicates that relatively little elimination of third chromosomes bearing lethals has occurred. Thus, lethals may be expected to have accumulated more rapidly in chromosome III, and the figures in generation 1 show that exactly this has happened.

It is clear in addition that the rise in mutation frequency caused by high temperature shown in table 1 is in reality greater than actually appears. The true increase is found by subtracting the control (generation 1) per cent in each case from the experimental total, and dividing the result by the average given in table 2. Thus, the heat appears to have produced about a three-fold increase in lethal mutation frequency in chromosome II, and a five- to six-fold increase in chromosome III. These results amply confirm the figures arrived at by Plough and Ives.[2]

Finally, an average of the lethals found in chromosomes II and III in generations 9–11 reveals a fact of extraordinary interest. The per cent of lethal bearing II chromsomes is 02.1 while that for similar III chromosomes is 02.5. *Cl B* tests of *X*-lethals on the same stock made early in 1936 gave a per cent of 01.1. Now the ratio of these percentages is almost exactly the same as the ratio of gene numbers as revealed by the salivary studies of Bridges and of Painter. This study of the frequency of autosomal lethal mutations *per generation* gives a much more accurate comparison than is possible from the data of Berg for chromosomes I and II only. It indicates

that the *rate of mutation per gene is approximately the same for each of the three chromosomes*, and that rate of mutation per chromosome is directly proportional to the number of genes it contains.

Another final point which our study proves conclusively for the first time is that a minimum rate of spontaneous mutation occurs even under the most constant environmental conditions. Generalizing from *Drosophila* this means that there is no such thing as a static species in nature. It gives further weight to the analysis of Wright[9] which requires a minimum genetic variation for evolutionary change, and it furnishes the experimental proof of the shifting genetic composition of the wild population of *Drosophila* at Gelendzhik recorded in the painstaking and important studies of Dubinin[10] and his co-workers.

Our complete reported recording in addition tests of frequencies of autosomal lethal mutations at various constant temperatures will appear at an early date.

[1] Muller, H. J., *Genetics*, **13**, 279 (1928).

[2] Plough and Ives, *Genetics*, **20**, 42 (1935). (Complete literature list.)

[3] Timofeeff-Ressovsky, Zimmer and Delbrück, *Nachrichten v. d. Gesell. d. Wissensch. z. Göttingen, N. F. I.*, 190 (1935).

[4] Ssackarow, W. W., *Genetica*, **18**, 193 (1936).

[5] Demerec, M., *Genetics*, **22**, 190 (1937).

[6] Plough and Holthausen, *Am. Nat.*, **72**, 185 (1937).

[7] Child and Plough, *Genetics*, **22**, 188, 189 (1937).

[8] Berg, R. L., *Ibid.*, **22**, 225 (1937).

[9] Wright, S., *Jour. Genetics*, **30**, 257 (1935).

[10] Dubinin, Heptner, Demidova and Djechkova, *Biologichesky Zhurnal* (*Russ.*), **5**, 962 (1936).

HAPLOID OVARIAN TISSUE IN DROSOPHILA

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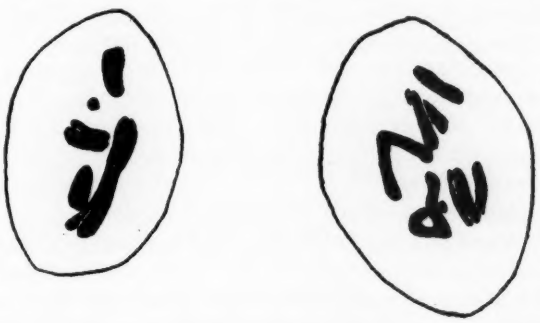
The question whether haploid tissue in *Drosophila* would be male or female was raised by Bridges (1921[1]), and its theoretical interest has been discussed in several papers (Schrader and Sturtevant 1923,[2] Bridges 1925a,[3] 1925b,[4] 1930,[5] Schrader and Hughes-Schrader 1931[6]). All haploid metazoa known to occur in nature are males, but Bridges' theory of sex-determination by genic balance requires that a haploid *Drosophila* be female. The recent studies of Whiting (1935[7]) and co-workers suggest that sex-determination in those species of animals in which the males are haploid may be in accord with the theory of genic balance.

Bridges sought a direct answer to the question by looking for haploid mosaic areas on specimens of *Drosophila melanogaster* of such genetic con-

stitution that haploid areas were expected to occur (though with a very low frequency) and to be recognizable as such if they did. He found, among others, two mosaic areas (Bridges, in Morgan, Bridges and Schultz 1930,[8] Bridges 1930[5]) which were anatomically female, and which, by the genetic characters which they showed, suggested strongly that they were haploid.

On the basis of the work of Stern (1936[9]) on somatic crossing-over and consequent segregation in flies similar in genetic constitution to those used by Bridges, an alternative, though perhaps less probable, explanation may be advanced in accordance with which these spots would have consisted of diploid tissue. But this explanation would require the occurrence of somatic double crossing-over in chromosomes II and III and single crossing-over in *X* at a very early stage in development, an event which, though possible, should be extremely rare. Furthermore, this explanation does not account for the smaller eye-facet and wing-cell size in that one of the two above cases in which these could be observed. The work of Bridges



appears to have shown more or less conclusively that haploid tissue in Drosophila is female.

The present paper describes a specimen of *Drosophila* in which tissue demonstrated cytologically to be haploid took part in the formation of a typically female organ, the ovary.

The individual in question came from a cross of *D. miranda* (Dobzhansky 1935[10]) females by *D. pseudoobscura* males. The miranda females were homozygous for Truncate, a sex-linked dominant (Duncan, unpublished). The males were wild type (race *A*, Texas strain). Heterozygous Truncate is readily distinguishable from normal, but occasionally overlaps homozygous Truncate. The mating produced, among many other offspring, a female which attracted attention because, unlike the majority of females from that cross, she showed rather distinctly the homozygous, not the heterozygous Truncate condition of the wings. It was then noticed that the individual cells composing her wings were much smaller

than normal. She was intermediate in body size between the largest and the smallest of the females that emerged from the same culture in the same two-day interval; in fact, aside from the characters mentioned above, there was little to distinguish her from the rest. The bristles on the dorsal side of the thorax were of about normal size. A careful measurement of her ommatidia showed that they were slightly, but not significantly, larger than those of two normal females from the same or a similar culture. However, the three right legs were distinctly smaller than their mates. No sex combs were present. The external genitalia were of the normal female type.

Within six hours after she was found she was observed to be moribund. This may have been due to over-etherization. She was dissected and acetocarmine preparations of her ovaries were made. The ovaries were normal in appearance, though, as was to be expected from her age, immature. The two ovaries together had thirty or more egg-strings.

The determination of the oogonial tissue as haploid is based on three metaphases and an anaphase in one ovary and one metaphase in the other, all showing one *V* and three rods, which, with the dot, would be the normal haploid complement of pseudoöbscura or miranda. In one of the above figures the dot was clearly seen. But, surprisingly, the follicle cells of the ovaries were not haploid. No figure was obtained in which their chromosomes could be accurately counted, but of those available the best, a telophase, showed approximately the diploid number.

In view of the asymmetry in the size of the legs, and in view of the fact that the ommatidia were of normal size, it seems likely that this female was a mosaic of haploid and diploid tissue. If so, the follicle cells might have come from a part of the early embryo which was diploid. The embryological relation of the follicle cells to the ovary of *Drosophila* is not clear. On the other hand, somatic doubling of the chromosome number is known to take place in certain tissues in *Diptera*, and might, in the present instance, have taken place during the development of the follicle cells, although it does not regularly do so.

Male and female parts show a high degree of autonomy in gynandromorphs of *Drosophila*, that is, genetically male and female tissue form male and female organs respectively, regardless of their surroundings. Since in the present case haploid tissue developed into a typically female structure, it seems highly probable that a *Drosophila* which was haploid throughout would be a female.

The author is indebted to the members of this department, and especially to Dr. C. B. Bridges, for helpful suggestions and criticism.

Summary.—A specimen of *Drosophila* is described in which tissue cytologically demonstrated to be haploid gave rise to oögonia.

[1] Bridges, C. B., *Science N. S.*, **54**, 252–254 (1921).
[2] Schrader, F., and Sturtevant, A. H., *Am. Nat.*, **57**, 379–381 (1923).
[3] Bridges, C. B., *Ibid.*, **59**, 127–137 (1925).
[4] Bridges, C. B., *Proc. Nat. Acad. Sci.*, **11**, 706–710 (1925).
[5] Bridges, C. B., *Science N. S.*, **72**, 405–406 (1930).
[6] Schrader, F., and Hughes-Schrader, S., *Quart. Rev. Biol.*, **6**, 411–438 (1931).
[7] Whiting, P. W., *Jour. Hered.*, **26**, 263–278 (1935).
[8] Morgan, Bridges and Schultz, *Yearb. Carnegie Instn.*, **29**, 352–359 (1930).
[9] Stern, C., *Genetics*, **21**, 625–730 (1936).
[10] Dobzhansky, Th., *Ibid.*, **20**, 377–391 (1935).

NOTE ON TRANSITIVE TRANSFORMATIONS

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A topological transformation T of a complete, separable, metric space Ω, without isolated points, is said to be transitive if any one of the following equivalent conditions is fulfilled:

(*a*) The complete sequence of images of some point under iteration of T and T^{-1} is dense in Ω.

(*b*) The images of some point under iteration of T, and also its images under T^{-1}, are dense in Ω.

(*c*) Any neighborhood overlaps any other one under some iterate of T.

(*d*) Ω cannot be divided into two disjoint invariant subsets both containing interior points.

(*e*) If E is any invariant set having the property of Baire,[1] then either E or its complement is of first category.

This type of transitivity has been considered, for example, by Birkhoff.[2] It is sometimes called regional or topological transitivity to distinguish it from metric transitivity, for which the characteristic property is:

(*e'*) If E is any invariant measurable set, then either E or its complement is of measure zero.

In view of the analogy between measurability and sets of measure zero on the one hand, and the property of Baire and sets of first category on the other,[3] the parallelism of conditions (*e*) and (*e'*) indicates that the above type of transitivity is to be regarded as the natural topological analog of metric transitivity.

Such transformations are of interest in connection with dynamical systems. If the flow induced in the phase space by the equations of the system admits a surface of section, then a necessary and sufficient condition that there exist everywhere dense streamlines is that the transformation of the surface of section be transitive.[2] Transformations which arise in this

way from Lagrangean systems admit an invariant integral, so that measure-preserving transformations are of special interest.[4] The existence of dense streamlines in the case of geodesic motion on surfaces of genus $p > 1$ has been shown by Morse[5] to follow from a hypothesis of "uniform instability," which is fulfilled on surfaces of negative curvature. Here we shall consider transitive transformations apart from their dynamical origin.

Various examples of transitive transformations are known. Besicovitch[6] recently defined one for the plane. The existence of one for a closed circular region has been mentioned by Kerékjártó[7] as an unsolved problem. The known cases of metric transitivity furnish examples, and likewise the results of Morse and Birkhoff. Birkhoff[2] has stated that the transitive case is probably to be regarded as the general case. The purpose of the present paper is to show that this conjecture can be made precise in the sense of category, provided we confine attention to measure-preserving transformations and thus to establish the general existence of transitive transformations. On the basis of a general lemma concerning metric groups, these results are used to prove a theorem concerning composition of transitive transformations.

Let Ω be any bounded closed region of euclidean r-space ($r \geqq 2$). The topological transformations $T,S, \ldots$ of Ω onto itself constitute a complete metric space $[H]$ with the metric

$$\rho(T,S) = \max_{x \text{ in } \Omega} (|Tx - Sx|, |T^{-1}x - S^{-1}x|).$$

Furthermore, the set of measure-preserving topological transformations of Ω is a closed subset of $[H]$, and is therefore itself a complete metric space which we denote by $[M]$.

The transitive measure-preserving transformations constitute all but a set of first category in $[M]$.

We shall indicate the principal steps in the proof. It suffices to show that the set of transformations such that no image of a neighborhood σ overlaps another neighborhood σ' is nowhere dense in $[M]$, σ and σ' being contained in the interior of Ω. For, by condition (c), every intransitive transformation corresponds to some such pair of neighborhoods; and by taking them as rational spheres, the set of intransitive transformations is represented as a countable sum of nowhere dense sets. The set under consideration being closed, it is sufficient to show that arbitrarily near any given T in $[M]$ one can find a transformation under which σ eventually overlaps σ'. This is accomplished by the following construction. Choose any point x_0 in σ and join any image $T^{k_0}x_0$ to σ' by a thin tube contained in the interior of Ω. Choose x_1 at distance nearly ϵ along this tube, and let it be so chosen that an image $T^{k_1}x_1$ lies very near x_1. This can always be done, since points having this recurrence property are dense.[8] Choose a similar point

x_2 nearly ϵ further along toward σ' and continue until a point x_p in σ' is reached. Consider the finite sequence $x_0, Tx_0, \ldots, T^{k_0}x_0, x_1, Tx_1, \ldots, T^{k_p-1}x_{p-1}, x_p$. Join each $T^{k_i}x_i$ to x_{i+1} by a connected neighborhood of diameter less than ϵ, the neighborhoods being taken disjoint and each containing no other points of the sequence. The manner in which the points were chosen makes it clear that this can be done, unless some $T^{k_i}x_i$ coincides with x_i. This case needs to be considered separately, but offers no difficulty. Now within each neighborhood define a transformation S so as to take $T^{k_i}x_i$ into x_{i+1} while leaving everything outside the neighborhoods fixed. For instance, let S be generated by a measure-preserving flow around closed tubes lying in each neighborhood. This S moves no point by more than ϵ, and is such that repeated application of ST takes x_0 into x_p. By choosing ϵ small, the transformation ST can be made to lie arbitrarily close to T, which completes the proof.

It follows at once that transitive measure-preserving transformations of Ω exist, and the proof indicates how they can be defined step by step.

It is an easy matter now to establish the existence of transitive transformations of any region. For we need only map its interior topologically on a bounded open region and consider the space of measure-preserving transformations of the closure of this bounded region which leave all boundary points fixed. The above argument applies to this space without change, and a transitive transformation corresponds to a transitive transformation of the original region.

There exist transitive transformations of any region of euclidean r-space ($r \geqq 2$).

Although the transitive transformations form a residual set in $[M]$, that is, all but a set of first category, they form only a nowhere dense set in $[H]$. For by an arbitrarily small change in a transitive transformation one can make an iterated image of a neighborhood σ lie within a closed subset of σ. Such a transformation is the center of a sphere of intransitive transformations in $[H]$. Thus, in the sense of category, transitivity is to be regarded as the general case among measure-preserving transformations, but is to be considered exceptional among all homeomorphisms.

The spaces $[M]$ and $[H]$ are not only complete metric spaces, but also groups. Furthermore, the group operations of product and inverse are continuous in the metric. Thus $[M]$ and $[H]$ are metric groups.[9] For such systems we have the following result.

If G is any metric group, and R is any residual set in G, then the group product $R \circ R = G$.

Let x be any element of G. The set $x \circ R^{-1}$ is homeomorphic to R, and therefore also residual. Hence the intersection $R \frown x \circ R^{-1}$ is likewise residual and therefore dense. Let r_1 be any one of its elements. Then $r_1 = x \circ r_2^{-1}$, that is, $x = r_1 \circ r_2$, where r_1 and r_2 belong to R. Applying

this lemma to the metric group $[M]$, we obtain the following theorem: *Any measure-preserving transformation of Ω can be expressed as the product of two transitive measure-preserving transformations. Either factor may be chosen arbitrarily from a set dense in $[M]$.*

In conclusion, it may be mentioned that in the case of three or more dimensions a modified method is available which enables one to prove the existence of transformations transitive in a stronger sense. Namely, every neighborhood has images which are ϵ-dense in Ω, ϵ being arbitrary. The consideration of this kind of transitivity was suggested to me by Dr. S. Ulam. It is hoped to discuss these matters in greater detail subsequently.

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[1] In the weak sense. That is, E can be obtained from an open set by adding and subtracting two first category sets. See C. Kuratowski, *Topologie*, I, Warsaw, 49 (1933).

[2] G. D. Birkhoff, *Acta Math.*, **43**, 1–119 (1922), esp. pp. 111–119.

[3] C. Kuratowski, loc. cit. See also W. Sierpinski, *Fundamenta Mathematicae*, **22**, 276–280 (1934).

[4] G. D. Birkhoff, *Trans. Amer. Math. Soc.*, **18**, 199–300 (1917), esp. p. 286.

[5] M. Morse, *Jour. Math.* (Liouville), (9), **14**, 49–71 (1935).

[6] A. S. Besicovitch, *Fundamenta Mathematicae*, **28**, 61–65 (1937).

[7] B. de Kerékjártó, *l'Enseignement Math.*, **35**, 297–316 (1936).

[8] Indeed they are all but a set of measure zero. G. D. Birkhoff and P. A. Smith, *Jour. Math.* (Liouville), (9), **7**, 345–379 (1928), esp. p. 355.

[9] S. Banach, *Théorie des Opérations Linéaires*, Warsaw, Chap. 1 (1932).

GROUPS IN WHICH EVERY SET OF INDEPENDENT GENERATORS IS A MAXIMUM SET

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A group G is said to have a maximum number set of independent generators whenever it has a set of independent generators which involves as many operators as there are prime factors in its order. It has recently been proved[1] that when G involves at least one such set of independent generators its Sylow subgroup whose order is a power of the largest prime number p which divides the order of G is invariant, abelian and of type 1^k. This Sylow subgroup may involve subgroups of order p which are non-invariant under G but when every possible set of independent generators of G involves a maximum number of operators, then every one of these subgroups of order p is invariant under G for if one of these subgroups would not be invariant under G then G would involve a set of independent generators involving an operator of order p which would generate this non-invariant

subgroup of order p. This operator and another operator of a possible set would generate a group whose order would exceed the product of the orders of these two generators and hence G would involve a set of independent generators which would not involve as many operators as there are prime factors in the order of G. In what follows G will be used to represent a non-abelian group in which every possible set of independent generators is a maximum set.

Every subgroup of the given Sylow subgroup of order p^k is therefore transformed into itself by every other operator of every possible set of independent generators of G. Since the order of G exceeds p^k every operator of this set whose order differs from p is non-commutative with each of the $p^k - 1$ operators of order p in the given invariant Sylow subgroup because every operator of G besides the identity must be of prime order. Such an operator therefore transforms every operator of this Sylow subgroup into the same power of itself since otherwise G would involve a non-invariant subgroup of order p. As the group of isomorphisms of the group of order p is cyclic the index of this power is prime. Hence there results the following theorem: *A necessary and sufficient condition that every possible set of independent generators of a group involves as many operators as there are prime factors in its order is that this order is either of the form p^k or of the form p^kq, p and q being prime numbers such that $p - 1$ is divisible by q. For every such order there is one and only one such group. In the former case the group is abelian and of type 1^k while in the latter case it is non-abelian but contains invariantly such an abelian subgroup, and every subgroup of this subgroup is invariant and each of its operators is transformed into the same power by the remaining operators of G.*

All the operators of G which do not appear in the given Sylow subgroup of order p^k are of order q, and the p^k subgroups of this order involve $q - 1$ sets of conjugate operators besides the identity. Whenever p is of the form $2^m + 1$ then G is either dihedral or generalized dihedral since it is non-abelian. For every prime number p and for every positive integral value of m there are therefore as many non-abelian groups which have the property that every set of independent generators is a maximum number set as there are distinct prime factors of $p - 1$. The only case when there is one and only one such group is therefore the one noted above when p is of the form $2^m + 1$ and hence $q = 2$. The largest value that q can have relatively to p results when $(p - 1)/2$ is a prime number and hence there is then a G of order $p^k (p - 1)/2$. The operators of a set of independent generators of G can be chosen in a number of ways which is equal to the order of the group of isomorphism of G and hence the group of order 2 and the identity are the only groups in which a set of independent generators can be chosen in only one way.

A group which has at least one set of independent generators, which in-

volves as many operators as there are prime factors in its order, contains at least as many distinct invariant subgroups whose common order is equal to the largest prime number which divides its order as there are independent generators in the Sylow subgroup whose order is a power of this prime number. This results from the fact that the operators of largest order in a maximum number set of independent generators may be so chosen that they generate the Sylow subgroup whose order is a power of the largest prime which divides the order of the group and when they have been so selected they generate subgroups which are separately invariant under all the operators of the set. A subgroup of such a group whose index is a power of the smallest prime number which divides the order of the group is invariant under the group. If the order of a subgroup is divisible by the orders of the k Sylow subgroups whose orders are powers of the k largest prime numbers which divide the order of the group as well as by the next largest prime number which divides this order it is not necessarily invariant under the group but there are at least as many such subgroups which are invariant as there are independent generators in the Sylow subgroup whose order is a power of this next largest prime number.

Since every set of independent generators of G is a maximum number set it results that the group of isomorphisms of G can be represented as a transitive substitution group of degree p^k in which the letters correspond to the operators of order q in G, G being non-abelian and of order p^kq. This substitution group involves invariantly a regular subgroup which is abelian and of type 1^k corresponding to the automorphisms of G in which every operator of its subgroup of order p^k corresponds to itself. That is, *the group of isomorphisms of the non-abelian group of order p^kq in which every possible set of independent generators is a maximum number set is the holomorph of the abelian group of order p^k and of type 1^k*. It may be noted that whenever $q > 2$ then G is conformal with groups in which there are sets of independent generators which involve a smaller number of operators than the number of the prime factors which divide the order of the group, but which involve at least one set of independent generators containing this maximum number of independent generators.

[1] Miller, G. A., these PROCEEDINGS, **23**, 281 (1937).

A SURVEY OF THIRTY-SIX THOUSAND SOUTHERN GALAXIES

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Communicated July 14, 1937

1. The first part of a systematic survey that should eventually include more than half a million galaxies has been completed with the measurement of the distribution and brightness of thirty-six thousand systems in the polar cap, south of declination $-60°$. About seven per cent of the sky is covered.

Because of the great number of faint galaxies that are discernible on long exposure plates made with the large photographic doublets, it is no longer advisable to measure and publish all possible details on each system. But the apparent photographic magnitude is of first importance; it is our most direct indicator of distance, and is the key to information on the space distribution of external systems. It will be determined for every nebula. Exact positions are of less importance, since there is little hope at present of measuring proper motions of such distant objects. For the preliminary discussions of metagalactic structure it suffices to use approximate positions for individual objects, and it will also suffice to measure dimensions and determine classes only in enough selected regions of the sky to get statistically dependable information on the general characteristics of faint galaxies. The more precise relationships of diameter to absolute magnitude and of magnitude and diameter to class can be better studied with objects of the fifteenth magnitude or brighter, and the classification itself can be more definitely elaborated for these same brighter objects. The combined measures of apparent magnitude and approximate positions of faint galaxies will yield information not only on metagalactic structure but also on the important problem of the total absorption of light in Milky Way regions and along the borders of the Galaxy.

2. Eighty-seven long exposure plates taken with the Bruce telescope have been used in the survey of the south polar cap. In establishing magnitude sequences and examining individual objects or special regions, a large number of supplementary long exposure plates have also been used, as well as numerous photographs of other series. Each of the eighty-seven plates has been examined to the limit of detection of nebulous objects. The number of objects falsely identified as external galaxies (perhaps three per cent) is believed to be practically balanced by that of objects that have been overlooked although above the plate limit. The false inclusions and exclusions are more common near plate edges, at the photographic limit of the plates and near the borders of the Milky Way. Less than one per cent of the galaxies of this polar cap have been previously recorded. There are

343 objects brighter than the fifteenth magnitude. They will be studied on small scale plates for the determination of total magnitudes, and on Bruce plates for detailed measurement and for the preparation of somewhat detailed descriptions.

The magnitudes of the individual objects have been twice measured. They are based on stellar sequences established for the most part by the star-count method. The magnitudes are estimated with a mean error of less than two-tenths of a magnitude, but fainter than the seventeenth magnitude the sequences are as yet provisional and the systematic error may approximate half a magnitude. I am indebted to Mrs. Muriel M. Seyfert

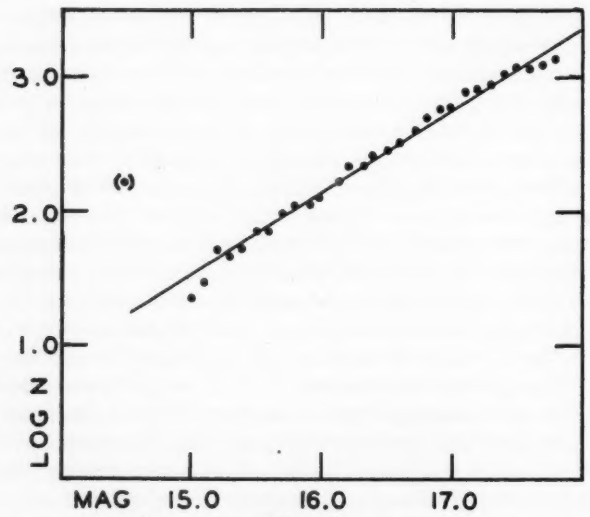

FIGURE 1

Relation of the logarithm of the number of galaxies to apparent magnitude for forty-two plates with galactic latitude higher than 25°.

for her expert assistance in the examination of the plates and in particular for her work in the most laborious part of the survey—the determination of the magnitudes.

3. In publishing the numerical details of the investigation, which will appear as one of the Tercentenary papers from the Harvard Observatory,[1] the distribution of the magnitudes is given for each square degree, as well as for each plate (thirty-five square degrees in area).

In figure 1 the density of galaxies over the whole polar cap is shown. The object of the diagram is to show the general features of the nebular distribution brighter than magnitude 17.5. For the average object on these

plates, this limiting magnitude corresponds to a distance of approximately thirty-five megaparsecs.* A few features of the density diagram are worthy of comment:

a. The long exposure plates actually cover completely only 88 per cent of the polar cap; for the brighter objects, however, additional plates have been used and the coverage is complete.

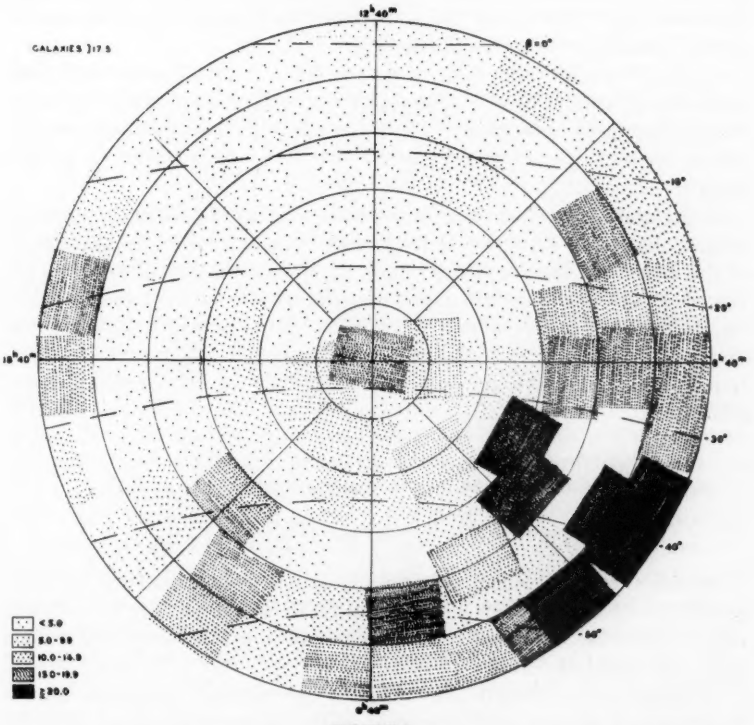

FIGURE 2

Distribution of galaxies brighter than magnitude 17.5 in the South Polar cap.

b. Both of the Magellanic Clouds fall within the polar cap and the regions they occupy were not surveyed for external galaxies because of the difficulty of distinguishing between nebulosities and star clusters in the Clouds and the external systems seen through them.

c. An exceptionally high density is shown by six nearly contiguous plates that cover an area extending from declination $-80°$ to the edge of the polar cap. In a subsequent communication a special report will be made on this metagalactic cloud which extends northward from the polar

cap about twenty-five degrees and apparently is a higher system comprising some forty or fifty thousand galaxies.

d. The effect of absorption in low galactic latitudes is indicated by the low density in the upper part of the chart. There is in fact a clear indication that the density over the whole of the polar cap (except for the metagalactic cloud referred to above) is considerably below normal for the sky in general. This deficiency may be due in part to details in metagalactic structure; but certainly in the lower latitudes it is almost wholly due to general absorption in the Milky Way.

e. In galactic latitude $-25°$, longitude 300°, there is an unusually high frequency of bright objects. The region is closely neighboring the galactic window[2] centered at latitude $-16°$, longitude 301°; plates falling just outside of the south polar cap show in this direction a high frequency of faint galaxies.

4. In figure 2 is shown the relation of the number of galaxies to apparent magnitude. Only 42 of the 87 plates have contributed to the calculation of the means for this frequency curve. The six rich plates have been excluded, as well as all plates in galactic latitudes lower than 25°, where galactic absorption seriously affects the results. For the area covered by the 42 plates, the number of galaxies per square degree brighter than magnitude 17.5 is 7. Determinations of this quantity for other regions are as follows:

Coma-Virgo (*H. A.* **88,** No. 1; *H. B.* 865)	21
Horologium (*H. A.* **88,** No. 5), including clusters	49
Horologium, excluding clusters	43
Longitude belt 110° (*H. A.* **105,** Paper No. 10)	28

Hubble's formula, which is based on Mount Wilson surveys, gives 21 galaxies to this magnitude limit. The result above for the Horologium region cannot be considered typical because of complications in that region with a metagalactic cloud.

In figure 2 the straight line indicates the expected frequency on the hypothesis of uniform space density. If the six rich regions are included with the 42 other plates, the pronounced deviation from the straight line relation of log N to apparent magnitude becomes obvious at photographic magnitude 16.5, which probably marks the brightness and implies the distance at which the metagalactic cloud becomes effective in the distribution of galaxies in the south polar cap.

5. To the limit of the present survey there are in the polar cap only a few physical groups of galaxies outside the strip covered by the metagalactic cloud. One falls on a rich plate and is itself populous enough to be treated as a significant cluster. It is located at longitude 276°, latitude $-31°$, only six degrees from the south celestial pole. So far as shown by

the available photographs it is composed of approximately 200 members, scattered somewhat irregularly over an area of 1.5 square degrees. On the assumption of three-tenths of a magnitude as possible space absorption, its distance is about forty megaparsecs.

* 115 million light years.

[1] *H. A.*, **105,** Paper No. 8, 1937.

[2] Shapley and Boyd, *H. C.*, **411,** 1936; *H. A.*, **105,** Paper No. 13, 1937.

THE GROWTH OF PLANT EMBRYOS IN VITRO. PRELIMINARY EXPERIMENTS ON THE RÔLE OF ACCESSORY SUBSTANCES

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The aseptic culture of plant embryos isolated from the seed dates back to the work of Brown and Morris,[1] Hannig[2] and Dietrich.[3] More recent contributions to our knowledge concerning the culture *in vitro* of excised embryos have been made by Tukey,[4] Brunner[5] and LaRue,[6] among others. It has been recognized by, for example, Ray[7] that the embryo culture technique offers a useful tool for biochemical investigations, and it has also been recognized[4,8] that it may be used as a practical measure to circumvent the abortion of embryos. It has, however, been found that in general the growth of the excised embryo, even upon a medium containing essential inorganic materials and sugar, is far less than that of normal intact seedlings. This has led to the suggestion[5] that "accessory growth factors" which are needed in minute amounts, are required by the developing plant as they are by the developing animal organism. The present work, as well as that of Kögl and Haagen-Smit,[9] furnish final proof that this is the case; that these accessory substances, although normally furnished by the seed, may be replaced to some extent by pure compounds added in small amounts to the embryo culture medium. These investigations, taken up early in 1936, are concerned particularly with orienting experiments undertaken with an ultimate view toward the elucidation of the nature and mode of action of these accessory growth factors. The embryo culture technique is here to be used as a tool in the "hormonal" analysis of plant development.

Materials and Methods.—Pea seeds of the "Perfection" variety were used for all of the principal experiments. A few of the preliminary experiments were done with the "Alaska" variety. Seeds were sterilized

by the usual technique of this laboratory (washed in 95% alcohol, soaked for 20 minutes in 0.1% $HgCl_2$) and then soaked in water for 6 (in some cases 9) hours in sterile Petri dishes. The embryos were then removed and cultured under sterile conditions. Infections were very rare. The basic culture medium was that which has been found to be highly satisfactory for the growth of excised pea roots[10] and contained the necessary inorganic salts with nitrate as the nitrogen source, as well as four per cent sucrose. This concentration of sucrose was found to be superior to lower concentrations for the growth of embryos as well as for excised roots. Agar medium was markedly superior to liquid medium. In the latter the growth of the shoot in particular was greatly diminished. Pyrex culture vessels were also superior to those of soft glass. It is worthy of note that while the embryos were able to make some growth either on liquid medium in Pyrex or on agar medium in soft glass, they were completely inhibited on liquid medium in soft glass. A comparison of the various types of possible culture vessels showed that flasks were somewhat superior to test tubes and hence Pyrex flasks were used for all of the cultures reported here. Twenty embryos were grown in each of the media tested in a single series. The individual values given in the tables below are thus each the mean from twenty plants. All cultures were grown in the dark and measured weekly with a flexible millimeter scale. At the end of four weeks each series was stopped and a final, more accurate measurement of root and shoot growth made.

Principal Experimental Results.—An attempt was first made to cultivate the excised apical meristem of the pea embryo. Such meristems grew, however, very poorly Additions of cotyledons, yeast extract, malt, peptone, etc., did not improve this growth but it was noted that those fragments which regenerated roots at their basal ends grew in the mean 2.5 mm. in two weeks, while those which did not form roots grew only 1.3 mm. in the same time. This was taken to indicate that roots *per se* are beneficial to stem growth. That this is actually true has been shown in other ways by Went.[11] That it is true in the present case is also shown by many other experiments in which the excised embryonic shoot grew less than one-half as rapidly as the entire embryo under the same conditions. This factor coming from the root and necessary for shoot elongation is apparently transported only through living tissue[11] and it was impossible to cause it to diffuse from excised shoot to excised root. It was also impossible to replace it by any of the extracts or substances which have been added to the medium. The further experiments were therefore made with the entire embryo.

Pea embryos when cultured on the basic medium attained a shoot length of 19 to 36 mm. in four weeks' time under the conditions used. In three series only did the growth on the basic medium exceed this amount and in

all three of these cases the relative response to added accessory factors was proportionately diminished. It seems reasonable to suppose that in these three series the embryos had for some unknown reason "mobilized" a larger portion of accessory substances from the cotyledon before excision.

Several series in which crystalline vitamin B_1[12] was added in varying amounts to the basic medium gave substantially identical results and as an example the measurements from a portion of a typical series are given in table 1. The effect of B_1 upon the root growth of the cultures was very marked, and it is possible that the effect upon shoot growth is therefore indirect. The optimum vitamin B_1 concentration was found to be roughly 0.13 γ per cc. and this concentration was used for the later combination experiments.

Vitamin C, also, caused a distinct increase in the growth rate of both root and shoot, as is shown in table 1. The concentration of 0.05 mg. per cc. was superior to 0.1 mg. or to 0.025 mg. per cc. and was also used in the later experiments.

TABLE 1

THE GROWTH *in vitro* OF EXCISED PEA EMBRYOS UPON MEDIA CONTAINING VITAMINS B_1 AND C AS ACCESSORY GROWTH FACTORS

MEDIUM	1 WEEK	2 WEEKS	3 WEEKS	4 WEEKS	
				STEM	ROOT
Control	10	18	25	34	85
B_1, 0.13/cc.	13	25	39	52	106
B_1, 0.013/cc.	8	18	30	44	104
Control	9	17	28	34	83
C, 0.05 mg./cc.	13	25	41	48	108

It has been found that a complex mixture of amino acids is necessary for the continued growth of excised roots[10,13] and such a mixture was therefore added to the basic medium as a co-growth factor for vitamin B_1. In none of the concentrations tested was the growth of the pea embryos increased over that brought about by vitamin B_1 alone. Inositol in conjunction with vitamin B_1 was also without effect.

Pantothenic acid,[14] however, was found to be a potent growth factor for these embryos. The optimum concentration of 0.15 gamma per cc. caused an increase in stem growth rate at least as great as that due to vitamin B_1 (table 2). The influence upon the root was less marked, however. Roots of embryos grown in pantothenic acid medium were longer than those in basic medium alone, but were neither as long nor as luxuriant as those grown on vitamin B_1 medium.

Folliculin was the fourth of the active substances investigated, and it also caused considerable increase in both root and shoot. Concentrations

TABLE 2

THE GROWTH *in vitro* OF EXCISED PEA EMBRYOS UPON MEDIA CONTAINING ACCESSORY GROWTH FACTORS ALONE AND IN COMBINATION

(Concentrations given in text)

MEDIUM	1 WEEK	2 WEEKS	3 WEEKS	4 WEEKS STEM	4 WEEKS ROOT
Control	10	21	24	34	80
Pantothenic acid	13	26	34	54	95
Vitamin B_1	9	20	33	51	109
Folliculin	13	31	40	51	102
B_1 + pant. acid	15	29	43	63	100
B_1 + pant. acid + C	15	39	51	68	141
B_1 + pant. acid + C + folliculin	16	40	54	65	149

of 0.13 to 0.67 gamma per cc. showed no considerable difference in growth-promoting activity, but were more beneficial than either higher or lower concentrations.

If two or more of the active accessory factors were added simultaneously to the basic medium the effect was greater than that of either alone but was not equal to the sum of the individual effects. This is shown clearly in table 2, where the results of series with vitamin B_1, pantothenic acid and folliculin are given, tested alone and in combination, as well as in combination with vitamin C. There are some irregularities but it is clear that there is a maximum average shoot length of about 65 mm. (although individual shoot lengths of as great as 95 mm. in four weeks have occasionally been recorded). The great effect upon root length of vitamin C in combination with B_1 and pantothenic acid is also worthy of note.

Leaf development was markedly less than that of the intact etiolated seedling and was not greatly influenced by any of the added accessory substances.

Discussion.—All of the four substances which have been shown to increase the growth of excised pea embryos are known to be natural plant products. Thus vitamin B_1, pantothenic acid and folliculin are known to occur in seeds, while vitamin C is formed during the germination process from sugars stored in the cotyledons.[7] It need not, then, occasion surprise that these substances apparently play a part in the development of the young plant, a conclusion which has been arrived at also by others. Thus Kögl and Haagen-Smit[9] in their work found an effect of vitamin B_1 upon the growth of the excised embryo similar to that recorded here. They have shown in addition that biotin, another normal constituent of the pea seed, produces much the same effect as does the related pantothenic acid used here. In their work just as in the present investigation, shoot growth in particular was increased by the bios II factor. Kögl and

Haagen-Smit have, however, reported that vitamin C was without influence upon the growth of the pea variety which they used. It seems not unlikely that varieties may differ in their ability to synthesize this substance for themselves; an ability which Ray[7] has shown that excised pea embryos do possess. In the present case vitamin C clearly acts as an accessory growth factor, in agreement with the results of von Hausen,[15] Havas[16] and Davis, Atkins and Hudson.[17] That folliculin exerts beneficial effects upon the growth of intact plants can no longer be doubted.[18] The present work showing that it is beneficial to the growth of excised embryos is also in accord with the findings of Kögl and Haagen-Smit.[9]

It is not surprising that the combination of several of the accessory growth factors should fail to yield additive growth responses. It is quite possible that as the growth rate is increased by the addition of successive factors, still other factors or conditions as yet unknown become limiting. It is also possible that the optimum concentration of one factor alone is not the same as the optimum concentration of the same factor in the presence of others. Both of these possibilities may well play a part in the determination of the upper growth rate limit shown in table 2. It is in any case clear that a "normal" growth of the excised embryo can be arrived at only by a long series of successive approximations.

[1] Brown, H., and Morris, G., *Jour. Chem. Soc. London*, **57**, 458 (1890).

[2] Hannig, E., *Bot. Zeit.*, **62**, 45 (1904).

[3] Dietrich, K., *Flora, N. F.*, **17**, 379 (1924).

[4] Tukey, H., *Proc. Amer. Soc. Hort. Sci.*, **32**, 313 (1934).

[5] Brunner, G., *Jahrb. wiss. Bot.*, **76**, 407 (1932).

[6] LaRue, C., *Bull. Torrey Bot. Club*, **63**, 365 (1936).

[7] Ray, S., *Biochem. Jour.*, **28**, 996 (1934).

[8] Laibach, F., *Zeit. Bot.*, **17**, 417 (1925).

[9] Kögl, F., and Haagen-Smit, A., *Zeit. physiol. Chem.*, **243**, 209 (1936).

[10] Bonner, J., and Addicott, F., *Bot. Gaz.* (in press).

[11] Went, F., "Specific Factors Other than Auxin Affecting Growth and Root Formation," *Plant Physiol.* (in press).

[12] The authors are greatly indebted to the Merck Co. for the supply of vitamin B_1.

[13] White, P., Paper presented before the Amer. Soc. of Plant Physiologists, Atlantic City, Dec. (1936).

[14] The authors are greatly indebted to Prof. R. J. Williams for the supply of pantothenic acid.

[15] von Hausen, S., *Biochem. Zeit.*, **288**, 378 (1936).

[16] Havas, L., *Nature*, **136**, 435 (1935).

[17] Davis, W., Atkins, G., and Hudson, P., *Ann. Bot., N. S.*, **1**, 329 (1937).

[18] Bonner, J., *Bot. Rev.* (in press).

THE EYE-STALK HORMONE AND RATE OF HEART BEAT IN CRUSTACEANS

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During a study of the color changes of the prawn *Hippolyte varians*, Gamble and Keeble (1900) observed that the pale or nocturnal phase was accompanied by a higher rate of heart beat (about 240 beats per minute) than the dark or day phase (150 beats per minute). They believed that these changes in rate of heart beat, color and other variable phenomena which remained in phase with day and night, even when animals were maintained under constant external conditions, were controlled by the nervous system. Since the independent discovery by Perkins (1928) and Koller (1928) of the eye-stalk hormone in crustaceans the results of numerous investigations indicate that the pigment changes of the body and eyes of crustaceans are probably all controlled directly by a hormone or hormones. The question of the number of hormones has not yet been answered. The source of the active principle from the eye-stalk has recently been discussed in some detail by Hanström (1937), and the chromatophorotropic substance has been shown by Abramowitz (1937) and others to resemble intermedin in many of its properties.

In the group Natantia, to which shrimps and prawns belong, the concentration of the red and yellow pigments has been shown to be due to the presence in the blood of what we may continue to call the eye-stalk hormone. The dispersal of these pigments has been shown to occur when the hormone is absent from the blood or perhaps below a threshold concentration. This suggests that the high rate of heart beat of *Hippolyte*, which accompanies the pale phase, may be due to the same hormone that affects the pigment cells, and that a lowering of the rate is due to a decrease in the amount of hormone in the blood. In order to test this theory an experiment was performed using the crayfish, *Cambarus virilis*, as a test animal since a supply of *Hippolyte* was not available.

The eye-stalk hormone is known to be non-specific for crustaceans, hence it may be obtained from one species and tested on another. This makes it possible to use as a source of supply almost any readily available stalk-eyed crustacean. Extracts of eyes and eye-stalks of *Palaemonetes vulgaris* were prepared by crushing them in cold blooded Ringer's fluid (20 eyes per cc.). Extracts of eyes and eye-stalks of *Cambarus bartoni* (10 eyes per cc., because of larger size) were prepared in a similar manner. Muscle tissue of crayfish in an amount roughly equivalent to the volume of the eyes was extracted to use as a control. The macerated eyes and muscle tissue were

boiled for one minute and the clear supernatant fluids decanted. The three extracts were then tested. Five of a series of eight tests were made by different persons who were unaware of the nature of the extracts and the results which might be expected. This doubtless accounts for some of the variations in the results, for slightly different methods of conducting the tests were used by different individuals. The procedure was to remove the carapace over the region of the heart and to dissect away the pericardium dorsally. The heart was allowed a few minutes to recover and ten determinations of the time for ten beats were made. The heart was then perfused with five drops of the extract of *Palaemonetes* eyes and after one minute the times for ten sets of ten beats were again determined. The heart was next washed by dropping on Ringer's fluid and allowed to recover its

TABLE 1

SHOWING THE AVERAGE TIMES FOR TEN BEATS OF HEART OF *Cambarus virilis*, ALSO THE RATE OF BEAT, BEFORE AND AFTER PERFUSION WITH EXTRACTS CONTAINING THE EYE-STALK HORMONE AND A CONTROL EXTRACT OF MUSCLE TISSUE. FOR A GIVEN TEST ON A PARTICULAR ANIMAL THE FIGURE GIVEN IS AN AVERAGE OF TEN DETERMINATIONS OF THE TIME FOR TEN BEATS

ANIMAL	NORMAL TIME FOR 10 BEATS	*Palaemonetes* E. S. H. TIME FOR 10 BEATS	CONTROL-MUSCLE EXTRACT TIME FOR 10 BEATS	*Cambarus* E. S. H. TIME FOR 10 BEATS
1	16.8 seconds	10.4 seconds	13.0 seconds	9.3 seconds
2	16.4	11.0	12.8	6.3
3	13.8	11.2	11.7	10.3
4	9.7	7.5	9.4	8.5
5	15.8	11.7	16.1	..
6	13.1	9.4	12.3	..
7	17.9	..	18.8	9.0
8	18.8	14.7	..	11.4
Averages	15.28 seconds	10.84 seconds	13.44 seconds	9.13 seconds
Beats/min.	39.2	55.3	44.6	65.7

original rate after which the control prepared from muscle tissue was applied and the rate of beat again determined. Following a second washing with Ringer's fluid the extract of the eyes of *Cambarus* was tested. For various reasons a test of one of the three extracts was omitted on each of four animals. The results are shown in table 1. In the first place it may be seen that the "normal" rate differs considerably for different animals. In the case of crayfish 4 it was very high as shown by the relatively short average time required for ten beats. However, in every instance the perfusion of the heart with the extract of eyes of *Palaemonetes* resulted in an increase in rate of beat and yielded an average rate of 55.3 beats per minute as compared with the "normal" rate of 39.2 beats per minute. It should be mentioned that washing with Ringer's fluid always caused an immediate but temporary increase in the rate of beat. This may have been due to re-

moval of products of metabolism, a change in ion balance (cf. Lindeman, 1928; 1929) or to other causes. The rate would soon return to the "normal" or to a slower rate; the increase seldom persisting for more than one minute.

In some animals the control extract of muscle tissue resulted in an increase in rate of beat which persisted for a considerable length of time. This may have been due to the presence of the hormone in the blood of the muscle tissue, which was not washed before extracting.

The greatest increase in rate of beat occurred after perfusion with an extract of the eyes of *Cambarus bartoni;* the average rate for the eight animals being 65.7 beats per minute; the ratio of the "normal" beat to this is 1:1.67. The ratio of rate of heart beat observed by Gamble and Keeble in dark *Hippolyte* to that in pale or nocturnal *Hippolyte* was 1:1.60.

The results from these few preliminary tests are sufficiently uniform to leave little doubt that there is a hormonal control of the heart beat in *Cambarus*. The situation in *Hippolyte* and in other crustaceans can be determined only by similar experiments. Whether the hormone that acts on the heart is identical with the hormone that affects eye pigments (Kleinholz, 1936), and body pigments, or whether there are several hormones in crustaceans as postulated by Brown (1935) remains to be determined.

The difference in the effectiveness of the extract of eyes of *Palaemonetes* compared with that of *Cambarus* is possibly a concentration effect but this is likewise a matter which may be determined only by further work. The crayfish heart is perhaps a better test object for studying concentrations than the pigmentary effectors, for with it more exact quantitative measurements may be made and satisfactory kymograph records of its beat are readily obtainable.

Abramowitz, A. A., *Biol. Bull.*, **72,** 344–365 (1937).
Brown, F. A., Jr., *Jour. Exptl. Zoöl.*, **71,** 1–15 (1935).
Gamble, F. W., and Keeble, F. W., *Quart. Jour. Micro. Sci.*, **43,** 589–698 (1900).
Hanström, B., *Kungl. Svenska Vetenskapsakad. Handl.*, **16,** 1–99 (1937).
Kleinholz, L. H., *Biol. Bull.*, **70,** 159–184 (1936).
Koller, G., *Zeit. vergl. Physiol.*, **8,** 601–612 (1928).
Lindeman, V. F., *Physiol. Zoöl.*, **1,** 576–592 (1928).
Lindeman, V. F., *Ibid.*, **2,** 395–410 (1929).
Perkins, E. B., *Jour. Exptl. Zoöl.*, **50,** 71–105 (1928).

THE SEMINAL VESICLES OF THE GOBY, WITH PRELIMINARY CHEMICAL AND PHYSIOLOGICAL STUDIES OF THE VESICULAR FLUID

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Seminal vesicles in the goby (Gobius minutus) were described in 1824 by Rathke.[2] In 1836[3] he described them in several other species of gobies, and in 1850 Hyrtl[4] described them in yet other species of these fish. Several other authors have described these organs more or less fully since these early times, the most recent of them, to the best of our knowledge being Eggert (1931),[5] who has given a full account of their structure, and a suggestion regarding their possible function in a large number of species of the Gobiiformes.

Nevertheless the existence of these organs appears to have been generally overlooked, while virtually nothing is known regarding their function. Several naturalists, to whose attention they have been brought, knew nothing of them, while we have found no mention of them in several of the standard works on comparative anatomy.[6]

This neglect has prompted the present brief contribution. The "mud-sucker," *Gillichthys mirabilis*, with which we worked, is about 200 mm. in maximum length and inhabits the mud flats and shallow channels of Mission Bay in San Diego, California, which are largely exposed at low tide. Regarding its breeding habits we have no certain information, but believe that it deposits its eggs in holes in the banks and bottom of the bays. Spawning time is probably in May and June, as we have found numerous males with motile sperm at this season, as well as females with large ovaries.

The anatomy of the vesicles in this species is similar to that described by Eggert (loc. cit.) in other gobies, and portrayed by him in his figures 1 and 6. Our study of their histology has been very brief. So far as it has gone, however, it corroborates the account given by Eggert, to whom the reader is referred for details.

Whether the vesicles of the gobies are homologous to those of other vertebrates is at present an open question. In salamanders (especially *Triton*) there are paired glands, which structurally appear to be very similar to those of the gobies. These, however, are appendages of the cloaca, not of the vasa deferentia, as in the latter. In mammals the seminal vesicles arise from the Wolffian duct and usually form an appendage of the vas deferens in the adult. They may exceptionally, however, open separately

into the urethra. Judging from the account of Eggert the vesicles in the gobies arise *in situ* as masses of solid cords in the posterior part of the genital ridge. Later these cords acquire lumina which communicate with one another and ultimately connect with the two vasa deferentia near their point of union. In origin, therefore, the seminal vesicles of the gobies differ somewhat from those of the mammals, while in structure there is no very close similarity between them.

The rarity of these organs in fish, and their absence in Amphibia (unless the cloacal appendages of a few salamanders mentioned above be their homologues, which is unlikely), reptiles and birds is, in our opinion, strong evidence against their homology with the seminal vesicles of mammals. And if, as is probable, the vas deferens of the teleost is a different organ from that of other vertebrates, the seminal vesicles of the former cannot be homologous with those of the latter. We believe, therefore, that they are organs *sui generis* in both classes. This also is the view of Eggert.

The function of the seminal vesicles of vertebrates is still unsettled. There are two main theories regarding it: (1) They may serve as a storehouse for sperm, and (2) as a gland whose secretion either prolongs the life of the sperm or aids it otherwise in fertilization.

In *Gillichthys* the vesicles appear to be secretory and not storage organs, as we have not found sperm in them, with the possible exception of the base of the tubules where the vas deferens enters the gland. There is nothing apparently to prevent sperm from entering the vesicles, however, and during or immediately preceding ejaculation they may do so. Rathke (1824) indeed found that the vesicles of *Gobius minutus* in May were partly filled with a "schneeweissen....Stoffe....der ganz das Ansehen des Saamens (Milch) hatte."

The fluid expressed from the seminal vesicles was pale yellow, turbid and rather viscous. The results of investigations of its physical and chemical properties are presented in table 1 (following page).

The chief substance in colloidal solution was clearly of protein nature as demonstrated by the characteristic charring odor, positive biuret, xanthoproteic and (faint) Millon's tests, precipitation by alcohol, by $(NH_4)_2SO_4$ and by colloidal iron.

The presence of any simple native proteins such as albumin, globulin, other serum proteins or histones was excluded by the non-coagulation by heat, the absence of precipitates in Heller's test, the xanthoproteic reaction or by the addition of faintly alkaline $CuSO_4$, and finally by ready resolution of the material in aqueous systems after being precipitated by alcohol and having stood in contact with the alcohol for overnight periods. Our material, unlike protamines or histones from fish sperm, gave no precipitate when added to solutions of other proteins such as albumin or edestin.

TABLE 1

PROPERTY OR CHEMICAL TEST	VALUE OR RESULT	REMARKS
pH (Beckman glass electrode)	7.15; 7.39	
Density (at 25.5°C.)	1.034 (cf. density of sea water at 25°C., 1.020)	
% Ca	0.19 (cf. in sea water, 0.042%)	
% Cl	0.24 (cf. in sea water, 1.934%)	
% total solids (i.e., dry weight)	13.3	
Boiling of neutral, acidic or alkaline solution	No coagulum	No native protein
Extraction test for lipochrome (carotenoid) pigments	Negative	
Addition of absolute alcohol to make 70–80%	Copious precipitate	
Filtrate		
Biuret test	Negative	
Filtered precipitate		
Macroscopic appearance	Very pale yellow; cheesy consistency	
Microscopic appearance	Masses of poorly defined strands, meshes and amorphous wads of translucent material	
Roasting	Charred readily with odor characteristic of burning protein	
Solubility	Insol. alc., ether; readily soluble in cold water, dil. or conc. acids or alkalies; yellowish solution (like Witte's peptone) in strong alkali, esp. when warmed	
Aqueous solutions		
Boiling	No coagulum	Not native protein
Addition of dil. or conc. acetic acid	No precipitate whether original fluid or redissolved precipitate thus tested	Not nucleoprotein nor mucoprotein
Heller's nitric acid test	Negative	Not complex protein
Xanthoproteic test	No precipitate, but ready yellowing and strongly positive test (orange to red-orange) with ammonia	Protein material
Biuret test	Strongly positive (rose-violet)	Protein material
Millon's reagent	Orange or yellow precipitate	Resembles test with Witte's peptone, unlike red when albumin is used
Molisch's test	Very faintly positive	*Trace* of carbohydrate (?)
Reduced sulfur test	Strongly positive	
Osmic acid test	No blackening	No fatty material

TABLE 1 [*Continued*]

PROPERTY OR CHEMICAL TEST	VALUE OR RESULT	REMARKS
Uric acid	Absent (no precipitate with acids)	
Jaffe's test for indican	Negative	
Nitroprusside test for glutathione	Negative	
Alkaline picrate test for creatinine	Negative	
Benedict's test for carbohydrates	Negative, both before and after first boiling in dil. acid	No free or combined carbohydrates
Addition of colloidal (dialyzed) iron	Copious precipitate	Protein material
Addition of $CuSO_4$ solution	Very faint precipitate (?)	Trace of primary proteose (?)
Action of half-saturated $(NH_4)_2SO_4$	Very little precipitate	Trace of primary proteose (?)
Action of more than half-saturated $(NH_4)_2SO_4$	Increasing amounts of precipitation beginning with about 55% saturation	Secondary proteoses
Treatment with saturated $(NH_4)_2SO_4$	Complete precipitation of all protein matter (biuret test on filtrate negative)	Secondary proteoses; no peptones or polypeptides
Addition of $CuSO_4$ to redissolved precipitate	No precipitation	No primary proteoses
Addition of egg albumin or edestin	No precipitate	Not a protamine

Primary proteoses (variously called protoproteoses and heteroproteoses) were absent or at most present in only faint traces as shown by the lack of precipitation by nitric acid in Heller's test and the xanthoproteic test, the failure of $CuSO_4$ to give an appreciable precipitate, and the appearance of only a very small amount of precipitate from a considerable mass of material by half saturation with $(NH_4)_2SO_4$. Peptones and polypeptides were considered to be absent because the filtrate from fully saturated $(NH_4)_2SO_4$ systems containing the protein consistently gave negative results in the biuret test.

The various positive tests for protein material, and the establishment of the absence of simple native proteins, protamines, conjugated proteins, such as nucleoproteins or mucoproteins, *primary* proteoses, peptones and peptides, leave the so-called *secondary* proteoses (deuteroproteoses) as the remaining possibility. Positive confirmation of the presence of these bodies would seem to be established by the peculiar set of physical and chemical properties exhibited, viz., the definitely shown protein nature of the material by several standard tests, the non-coagulability by heat, strong acids, $CuSO_4$ or half saturation with $(NH_4)_2SO_4$ (save for a trace of precipitate), the ready precipitation with alcohol, ready solubility of the precipitate in water, salt solutions, acid or alkali, and finally the complete

precipitation with fully saturated $(NH_4)_2SO_4$. The chief material of the seminal vesicular fluid is thus concluded to be a non-blood protein of the nature of secondary proteoses (possibly containing thioproteoses as shown by the strong sulfur test) with traces of primary proteoses.

We have not been able to test the effect of the secretion on the fertilizing power of the sperm, since we have not been able to obtain egg-laying females. We have, however, repeated (with certain modifications to be noted later) the experiments of Eggert to test its effect on the length of life of the sperm, as a result of which we find, contrary to Eggert, that it has no appreciable effect. In 19 experiments, treated sperm lived on an average from 2 hours 6 minutes to 3 hours 31 minutes, as compared with an average of from 2 hours 5 minutes to 3 hours 20 minutes of 22 controls. While these results indicate a slight advantage in favor of the experimental fish, the difference is too small to have any significance in view of the number of experiments involved and their large variations.[7]

The details of these experiments are set forth in table 2 and we need add only a few words descriptive of our methods and the precautions employed to prevent contamination of the controls.

Seven sets of experiments were run, each from a single fish, with from one to five experiments in each set.

Sperm obtained from the testes, without addition of sea water or Ringer's fluid, are inactive, as has been found by previous workers, but on addition of either of these fluids they quickly become active. In our experiments small pieces of testes were removed from the experimental fish and quickly teased in a drop of sea water. For the controls sea water[8] alone was used, while for the experiment a small amount of secretion obtained from the vesicle of the same fish was added to the drop of sea water. All glassware and instruments were carefully cleaned and dried, but were not sterilized. After preparing the controls the instruments were cleaned and wiped again before preparing the experiments. In Series 6–7 instead of distilled water a drop of $N/10$ KOH was placed in the slide well to absorb the CO_2 generated by the sperm, and the sea water was buffered, in order to keep the pH of the sperm suspension as nearly constant as possible, since when not buffered the pH apparently dropped from 8.1 to about 6.3.[9] It was not found, however, that these precautions had any material effect on the length of life of the sperm. If they had any effect it was deleterious.

In two experiments of Series 7 ovarian extract[10] was added to a suspension of sperm and vesicle extract to determine whether in the presence of the former the latter would have more effect than when used alone. Two controls were run with egg extract only added to the sperm.

We considered cessation of all movement in the suspensions as the end point of the experiments, although cessation of activity proceeds gradually

in *Gillichthys*, and not suddenly as Eggert says occurs in *Gobius panizzae*. In one of our experiments, in fact (Ser. 1, exp. 1), a few sperm were motile for several hours after the majority had ceased to move, whereas in *G. panizzae* cessation of movement occurs in five minutes or less.

While the results of these experiments are naturally not identical, the general conclusion to be drawn from them is that, with the conditions under which we worked, the extract of the vesicles in *Gillichthys* is ineffectual in prolonging the life of the sperm. In Series 1, to be sure, such

TABLE 2

SHOWING SURVIVAL TIME OF SPERM TREATED WITH SECRETIONS OF THE SEMINAL VESICLES COMPARED WITH CONTROLS

Where two times are given it means that the survival period was intermediate between these two. Sea water was the menstruum used in every case except as noted. In Series I–III the hanging drop cultures were sealed over dry wells. In Series IV and V a drop of distilled water, and in Series VI and VII a drop of 0.1 *N* KOH was put in the well. In Series VI the sea water was buffered with $NaHCO_3$ (0.1%) and in VII with $Na_2HPO_4.2H_2O$ (approx. 0.02%).

SERIES	NO. OF EXPTS.	TIME	NO. OF CONTROLS	TIME
I	1	Over 8h. 30m.	1	2h. 17m.–5h. 30m.
II	1	Less than 4h.	2	Less than 4h.
	2	4h. 30m.–5h. 30m.	1	4h. 50m.–5h. 40m.
III	1	1–2h.	1	1–2h.
	2	2–4h.	1	2–4h.
	1[11]	45m.–1h. 30m.	1	Over 4h.
	1[11]	1h. 45m.–3h. 45m.	1[11]	1h. 45m.–3h. 45m.
IV	3	3–4h.	2	2–3h.
	1	3h. 45m.–4h. 20m.	2	3–4h.
			1	More than 4h. 20m.
V	2	1h. 30m.–3h.	3	1h. 50m.–3h. 10m.
	1	3h.–3h. 40m.	1	3h.–3h. 30m.
VI	1	1h. 25m.–2h. 15m.	4	1h. 40m.–2h. 30m.
	3	2h. 25m.–3h. 10m.		
VII	2	Less than 1h. 40m.	1[12]	1h. 40m.–2h. 15m.
	2[12]	Less than 1h. 35m.	1[12]	2h. 15m.–2h. 45m.
			1	More than 3h. 4m.
			1	1h. 40m.–2h. 20m.

Legend: h., hours; m., minutes.

an effect was apparent, but this single experiment is insufficient to contradict the results of the others. How this *one* contradictory result is to be explained we cannot say. Nor can we explain the difference between our own results and those of Eggert. The latter gives few details regarding his experiments so that it is difficult to compare them with our own. It is possible that the sperm we used was not fully ripe, even though actively motile, and that the extract would be effective only with fully ripe sperm. It is also possible that the specimens we used were not

healthy ones, since there were frequent deaths in the aquarium from which these fish were taken. We chose only specimens which appeared healthy, however; the sperms were very active when first isolated in every case, and their survival was much greater in our experiments (from 2–4 hours) than in those of Eggert (from one-half to one hour). Our material and technique, therefore, were apparently at least as satisfactory as his.

In order to satisfactorily determine the function of these organs it will evidently be necessary to obtain spawning fish.

Summary.—The seminal vesicles of the goby, discovered by Rathke in 1824, but since then overlooked by most writers, are structures *sui generis* and not homologous to those other vertebrates. Their function is uncertain, but it is probably primarily secretory and only secondarily, if at all, for storage of sperm. They contain a yellow, viscous fluid, which, when precipitated by alcohol, reveals a large content of secondary proteoses with traces of primary proteoses.

[1] In this work, the morphological and physiological studies were made by R. T. Young, the chemical investigations conducted by D. L. Fox.

[2] Rathke, H., "Beiträge zur Geschichte der Thierwelt. II. Über die Geschlechsteile der Fische." *Neueste Schrift. d. Naturforch. Ges. in Danzig*, **1**, 3 (Cit. Eggert) (1824).

[3] *Ibid.*, "Bildungs- und Entwicklungsgeschichte des Blennius viviparus oder des Schleimfishces." Abhandl. z. Bildungs- u. Entwicklungsgeschichte d. Mensch u. d. Tiere. Teil II. Leipzig (Cit. Eggert) (1836).

[4] Hyrtl, J., "Beiträge zur. Morphologie der Urogenitalorgane der Fische," *Deutsch. Kaiserl. Akad. Wiss. Wien. Math.-Naturw. Kl.*, **1** (Cit. Eggert) (1850).

[5] Eggert, B., "Die Geschlechtsorgane der Gobiiformes und Blenniiformes," *Zeit. wiss. Zool.*, **139**, 249–558 (1931).

[6] Gegenbaur's Vergleich. *Anat. d. Wirbeltiere* (1901), Weidersheim's Vergleich. *Anat. d. Wirbeltiere* (1909), Goodrich's Studies on the structure and development of vertebrates (1930), Ihle, *et al.*, Vergleich, *Anat. d. Wirbeltiere* (1927), etc.

[7] See table 2.

[8] In one control and two experiments Ringer's fluid was used.

[9] Due to the small amount of fluid available, which was barely sufficient to wet the electrodes in the Beckman meter, some uncertainty attaches to this reading.

[10] From an ovary which was evidently involuting.

[11] Ringer's fluid used instead of sea water.

[12] Ovarian extract was added to the seminal vesicle extract in expt., and sea water in controls.